

polynucleotides or other biomolecules having various reactive groups such as amino groups, sulfhydryl groups, or phosphothionate groups. For example, an aminoalkyl silane is immobilized on a substrate to provide a surface having attached functional groups with terminal amino groups. In a second step, the functionalized substrate is reacted with a maleimide carboxylate to provide a reactive maleimide group attached to the surface linker group. The reactive maleimide groups are used to attach a polynucleotide. However, this conventional method typically results in undesirable residual amino groups.

[0101] In one embodiment of this invention shown in **FIG. 6B**, a maleimide silane is used to functionalize a substrate in a one step silanization reaction. In a maleimide silane, the reactive maleimide group is separated from the silane group by a spacer group which may have, for example, any one of a variety of carbon numbers to provide a variety of lengths of spacer chains between the two reactive groups. Substrates functionalized in this reaction have reactive maleimide groups immobilized on the surface, and no residual amino groups. The reactive maleimide groups on the surface may be reacted, for example, with sulfhydryl functionalized polynucleotides or other biomolecules to be attached to the surface. Unreacted maleimide groups may be blocked with various sulfhydryl-containing reagents, to provide a substrate with attached polynucleotides or other molecules, useful as probes. In further embodiments, the spacer may be one of a variety of polymers or chemical chains, for example, a polyethylene glycol. Various reagents may be added to the sulfhydryl functionalized reactant to prevent cross linking or other coupling of the molecules, such as a reagent to prevent disulfide bond formation.

[0102] A substrate may be functionalized in a one step silanization reaction with the maleimide silane by a variety of techniques. For example, solution phase reaction of the maleimide silane with the substrate surface may be used. Alternatively, vapor phase deposition of the maleimide silane on the substrate surface may be used. In further embodiments, the substrate may be cured after reaction of the maleimide silane with the surface. Curing may be performed over a wide range of temperatures for a period as long as one day or longer.

[0103] C. Light Activation of Arrays

[0104] In further embodiments, the substrate may be chemically functionalized with surface-immobilized protected functional groups, where the protected functional groups are capable of being activated by absorption of light to provide reactive activated functional groups. The activated functional groups may be used to attach molecules, cells, or biomolecules to the surface. A mask or fiber optic bundle may be used to create a substrate having interspersed regions of activated and non-activated functional groups by irradiation of the substrate with light through the mask or fiber optic capillary bundle. The size, features, and morphology of the regions having activated functional groups are precisely controlled by the mask or fiber optic bundle. Biomolecules may be delivered to the surface and react to bind to the activated functional groups. Thus, the surface can be patterned to provide regions with bound biomolecules of precisely controlled size and morphology, regardless of the size or features of the region where the biomolecules were initially delivered to the surface.

[0105] In one embodiment shown in **FIG. 6C**, an aldehyde silane as discussed previously is used to functionalize the substrate by a silanization reaction. The aldehyde silane includes a photoreactive or photolabile group which, upon irradiation of the substrate, is cleaved from the surface immobilized silane, leaving a reactive aldehyde group attached to the substrate. The photolytic reaction can also be controlled by introducing a solvent to the substrate surface, or, for example, by introducing one or more of various photosensitizer or photoinhibitor agents to the surface.

[0106] Other methods for binding biomolecules, such as polypeptides and proteins, nucleic acids, carbohydrates, lipids, and metabolic products or other ligands, as well as larger biological assemblies such as viruses, subcellular organelles, or even cells, to solid supports are well-known and characterized in the art. Generally, a biomolecule or other structure may be immobilized either covalently or non-covalently to the support; either type of binding may require modification of the biomolecule, or the support, or both. In some cases, a binding pair, such as avidin/streptavidin and biotin, is used and one member of the pair is linked to the solid support while the other is linked to the biomolecule.

[0107] For nucleic acids, there are many techniques available and in common use, including covalent immobilization with or without pretreatment of support and/or nucleic acid (see, e.g., U.S. Pat. Nos. 6,048,695; 5,641,630; 5,554,744; 5,514,785; 5,215,882; 5,024,933; 4,937,188; 4,818,681; 4,806,631; Running, J. A. et. al., *BioTechniques* 8:276-277 (1990); Newton, C. R. et al. *Nucl. Acids Res.* 21:1155-1162 (1993)), non-covalent immobilization (e.g., U.S. Pat. No. 5,610,287), immobilization via avidin/streptavidin-biotin (e.g., Holmstrom, K. et al., *Anal. Biochem.* 209:278-283 (1993)). One very common substance used to prepare a glass surface to receive a nucleic acid sample is poly-L-lysine. See, e.g., DeRisi, et al. *Nature Genetics* 14: 457 (1996); Shalon et al. *Genome Res.* 6: 639 (1996); and Schena, et al., *Science* 270: 467 (1995). Other types of pre-derivatized glass supports are commercially available (e.g., silylated microscope slides). See, e.g., Schena, et al., *Proc. Natl. Acad. Sci. (USA)* 93: 10614 (1996).

[0108] For proteins, general techniques may be found in *Methods in Enzymology*, Vol. 44 (Immobilized Enzymes Edited by Klaus Mosbach, 1977); Vol. 135 (Immobilized Enzymes and Cells, Part B, Edited by Klaus Mosbach, 1987); Vol. 102 (Hormone Action, Part G: Calmodulin and Calcium-Binding Proteins, Edited by Anthony R. Means and Bert W. O'Malley, 1983); Academic Press, New York. Methods of covalent binding of proteins to supports may be found in, e.g., U.S. Pat. No. 5,602,207 and Zhang and Tam, Thiazolidine formation as a general and site-specific conjugation method for synthetic peptides and proteins, *Anal. Biochem.* 233: 87-93 (1996), Support and method for immobilizing polypeptides.

[0109] Methods developed for the binding of antibodies to glass supports are of use, not only to bind antibodies, but other proteins as well. See, e.g., U.S. Pat. No. 5,646,001; Bhatia et al., Use of thiol-terminal silanes and heterobifunctional crosslinkers for immobilization of antibodies on silica surfaces, *Anal. Biochem.* 178:408-413 (1989); Yanofsky et al., High affinity type I interleukin 1 receptor antagonists discovered by screening recombinant peptide libraries,